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Slab-gel electrophoresis

Electrophoretic analysis of proteins and nucleic acids on slab gels is standard laboratory practice today. However, 30 years ago gel electrophoresis was relatively new, and the analysis of complex protein mixtures, which we now take for granted, was not widely used. One of the drawbacks was that most analyses were done in glass tubes. It was tricky to get a set of gels with identical length, hard to remove the gels from the tubes once they had been run, difficult to compare the patterns between samples, and analyzing radioactive patterns was not straightforward. Here, I describe the background and impetus for developing a slab-gel system that rapidly gained wide use and whose basic design is still widely used today.

In December 1964 I took up a staff position in the Biology Department of Brookhaven National Laboratory (Upton, NY), fresh from postdoctoral research in Dale Kaiser's lab at Stanford Medical School (Stanford, CA). I planned to continue work I had done at Stanford using analytical ultracentrifugation to study conformational changes in DNA[1], and to initiate genetic and biochemical studies of bacteriophage T7, using techniques for genetic analysis that I had acquired in Dale's lab. I had been introduced to T7 by Bob Sinsheimer during my graduate work at Caltech (California Institute of Technology, Pasadena, CA). The simplicity that had attracted me to T7 DNA for physical–chemical studies also made T7 attractive as a model system for studying DNA interactions inside cells, including DNA replication, recombination, mutagenesis, control of gene expression and viral assembly.

At Brookhaven, I began to isolate amber mutants of T7, develop a T7 genetic map, and look at the effects of different T7 mutants on DNA replication and the ability to make phage particles. During this period, Jake Maizel, a friend from graduate school, was working at the Albert Einstein College of Medicine (New York, NY). There he developed, introduced and promoted the use of sodium dodecyl sulfate (SDS) in polyacrylamide electrophoresis as a simple and powerful way to dissociate proteins into individual chains and separate them according to size. He and his colleagues at Einstein were applying the method very successfully to analyze poliovirus and adenovirus proteins[2–4], so we invited Jake to Brookhaven to look at T7 proteins with his new technique. We also wanted to explore gel electrophoresis for separating nucleic acids. Jake was keen on the potential of gel electrophoresis and would joke that gels would make obsolete the analytical ultracentrifuge I was using daily to study DNA. As it turned out, I stopped using the ultracentrifuge only a few years later, and removed it from my lab to make space for running gels!

Jake came out to Brookhaven for the summers of 1967 and 1968. SDS gel electrophoresis proved to be a beautiful way to analyze the proteins of purified phage particles, visualize the time course of protein synthesis after phage infection, and identify the proteins specified by different T7 genes. The amber mutants I had isolated were ideal because they cause protein chains to terminate at the site of the mutation and, because SDS gel electrophoresis separates protein chains by size, the band at the position of the wild-type protein disappears in the mutant. My technician Olive Ritter and I would purify wild-type and mutant phage particles, or label cultures with radioactive amino acids after infection; Jake would electrophorese the samples on SDS gels and either stain them with Coomassie blue (for purified phage particles) or prepare autoradiograms (for radioactively labeled samples). Jake imaged the purified phage particles by electron microscopy, to correlate individual proteins with structural elements.

The results were very exciting, as we rapidly identified the proteins of the phage particles, followed the time course of protein synthesis during infection, identified the proteins specified by different genes, and demonstrated the critical importance of a single T7 gene in controlling all late gene expression (soon identified by others as T7 RNA polymerase)[5,6]. Jake was using an apparatus he had developed for electrophoresis of polyacrylamide gels in as many as 12 glass tubes in parallel[7]. After electrophoresis, the gel cylinders were extruded for staining or autoradiography. Processing for autoradiography was somewhat complicated[8]. The gel cylinders were sliced lengthwise into about four slices, in a device that allowed thin wires to pass through them, similar to a slicer for hard-boiled eggs. A center longitudinal slice from each gel cylinder was carefully laid out on filter paper, aligning slices from different tubes next to each other. The slices were pressed against the filter paper and dried with heat under vacuum, preserving their original shape and making them suitable for autoradiography[7].

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While Jake was in Brookhaven, he did all the electrophoresis and processing of the gels for the samples we analyzed. When he returned to Einstein, we took samples to him, but that was not very convenient and became impossible when, in 1969, he left for a sabbatical at the MRC Laboratory of Molecular Biology (Cambridge, UK). It was clear that we needed to do the electrophoresis and autoradiography ourselves. At just this time, Gary McGovern, an experienced technician, came to work with Olive and me. Gary arranged for the Brookhaven workshops to make the necessary electrophoresis and drying equipment, and soon had us up to speed in processing and analyzing gels.

When we started doing the electrophoresis and autoradiography ourselves, we confronted the difficulties in using the method first-hand. The electrophoresis was reproducible, but comparisons of samples between different gel tubes were not always straightforward, and subtle differences could be difficult to interpret. Nevertheless, we quickly acquired the requisite skills and soon many tube-gel electrophoresis systems were in use around the department.

Because autoradiography was proving so powerful for analyzing protein synthesis during T7 infection, we began to generate more and more samples. Processing all the gels became something of a chore, and we began to consider whether slab gels might offer substantial advantages. If slab gels worked, many samples could be processed conveniently in parallel, comparisons among samples should be better, and the surface noise apparent in autoradiograms of sliced gel cylinders might be substantially reduced or eliminated. Others were also experimenting with slab gels around this time[7].

As we were considering possible configurations, Bill Siegelman, a colleague in our department, pointed out the 1968 paper by Reid and Bieleski[9], 'A simple apparatus for vertical flat-sheet polyacrylamide gel electrophoresis'. This paper described a plexiglas apparatus with lower and upper buffer chambers that allowed gels prepared between glass plates to be used for electrophoresis, under essentially the same conditions that we were using for tubes – vertically and with the gel in direct contact with the electrode buffer at both ends. This configuration appealed to us. To improve convenience and throughput we modified their design to introduce a long notch in place of a slot in the interior glass plate and upper buffer chamber, and we tried

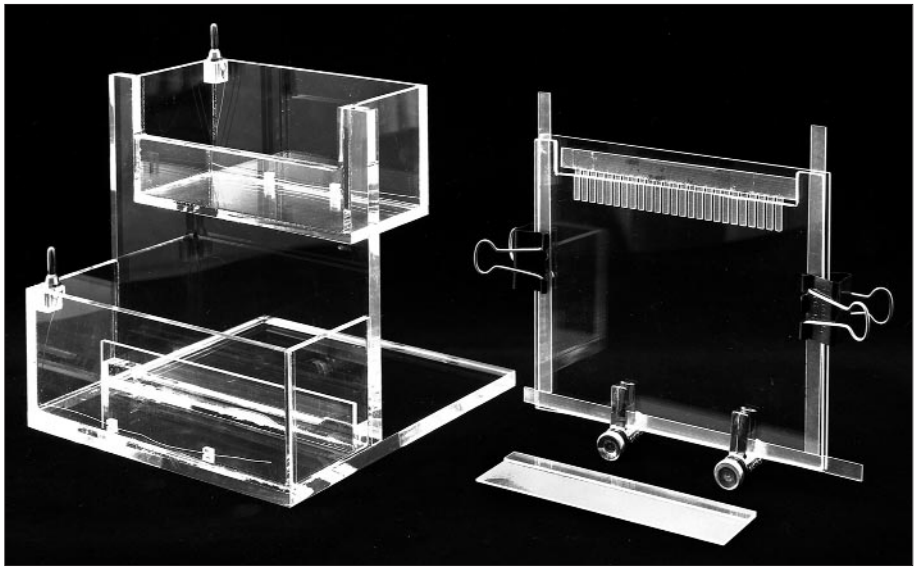

Figure 1

Slab-gel apparatus. The glass plates are shown assembled with side and bottom lucite spacers in place, and the comb inserted at the notch. Reproduced, with permission, from Ref. 13.

lucite combs to form initially 13 and ultimately 25 sample wells (Fig. 1). We had our design built in the Brookhaven electronics workshop and tried it out in November 1970. The configuration proved easy to use and the slab gels worked beautifully for both autoradiography and staining. Within weeks, slab gels had almost completely replaced tube gels in our lab. Figure 2 shows an example of the types of results we obtained.

During his sabbatical at Cambridge, Jake and Uli Laemmli successfully applied a discontinuous buffer system to SDS gel electrophoresis, something Jake had tried earlier but put aside because of problems in interpreting and compensating for unusual stacking effects[10]. Results showing the improved resolution given by the discontinuous buffer system were reported by Laemmli in 1970 (Ref. 11). (A projected joint paper describing the method never materialized.) The discontinuous buffer system gave excellent results in the slab gels, and soon we were using it almost exclusively. Subsequently, we found that a gradient of polyacrylamide concentration, together with the discontinuous buffer system, provided good resolution of protein bands over the entire molecular-weight range on a single slab gel.

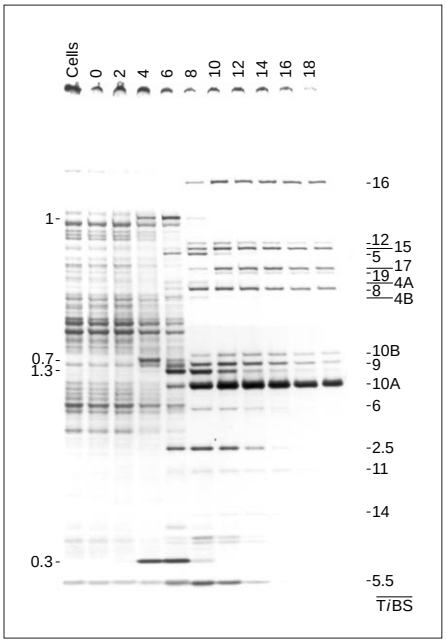

Figure 2

Time course of protein synthesis during T7 infection. The gene numbers of prominent T7 proteins are indicated to the side of the patterns. Reproduced, with permission, from Ref. 22.

Use of our slab-gel design spread quickly. I showed some slab-gel results at a seminar at Cold Spring Harbor Laboratory in December 1970, and Ray Gesteland began using it for work on *in vitro* translation. I, of course, told others and, by May 1971, I was distributing plans for the apparatus together with recipes and procedures for using it for SDS polyacrylamide electrophoresis. Many visitors to the Cold Spring Harbor Laboratory saw or heard about the system and took away copies of the plans and procedures. People began asking if the Brookhaven workshops could make the apparatus for them. This was not easily done, but Bob Dillingham, head of the Brookhaven workshop that built the apparatus for us, had a small commercial enterprise, the Aquebogue Machine

and Repair Shop, where he started to build the apparatus for people who requested it. My first published description of results obtained with the slab gels was in an article on T7 in 1972 (Ref. 12). A full description, including the use of gradient gels, was published in 1973 (Ref. 13). I continued to receive requests for the detailed plans and procedures into the 1980s, and sent out hundreds of copies. These requests finally dropped off as commercial systems, mostly based on our design, became widely available.

The great success in analyzing T7 proteins meant that Jake and I never did much exploring of separations of nucleic acids in the summers he was at Brookhaven. However, shortly after we started using slab gels, we began to use polyacrylamide or agarose/polyacrylamide composite gels to analyze T7 RNAs made during infection[13]. The discovery of restriction enzymes that cut DNA at specific nucleotide sequences[14,15] provided a great stimulus for the use of electrophoretic separation of DNAs in agarose gels, which could resolve much larger DNAs than polyacrylamide gels. Much of the early work with agarose gels used tube gels and, again, slab gels had obvious advantages. By 1973, Mike McDonell, a PhD student, and Martha Simon, a research associate, had joined our group and begun using agarose gel electrophoresis to analyze T7 DNA and its restriction fragments. The vertical slab-gel configuration proved unsuitable for agarose gels, and we soon developed a horizontal configuration that supported distortion-free electrophoresis at low agarose concentrations and allowed sample wells to be formed without tearing the gel[16]. We developed a more convenient apparatus shortly after, and offered plans but did not publish a description[17]. Again, we sent out hundreds of copies of plans and procedures and, again, the Aquebogue Machine and Repair Shop marketed copies. Other groups developed convenient designs for horizontal agarose slab gels around this time, and eventually horizontal designs became available from many commercial sources.

Other important techniques that have taken advantage of slab-gel electrophoresis include two-dimensional separations of protein mixtures, introduced by O'Farrell[18]; blotting procedures for identifying specific nucleic acids in complex mixtures, introduced by Southern[19]; and DNA sequencing by the method of Sanger and Coulson[20], or of Maxam and Gilbert[21] (although sequencers based on capillary electrophoresis are now taking over in high-throughput DNA sequencing centers). Slab-gel electrophoresis systems for a variety of uses are now widely available, and any lab that works with proteins or nucleic acids is almost sure to have access to one or more home-built or commercial system.

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SDS polyacrylamide gel electrophoresis

In 1961 I joined Harry Eagle's newly created Department of Cell Biology at the Albert Einstein College of Medicine in the Bronx, New York, as an Assistant Professor. While studying poliovirus and adenovirus capsid proteins by biophysical methods, I was surprised to find four proteins[1,2] after dissociating poliovirus and doing electrophoresis in 8 M urea-containing polyacrylamide tube gels[3]. This was unexpected because simple icosahedral RNA viruses were proposed, on theoretical grounds, to have capsids with a single protein[4,5] and several small icosahedral plant viruses were known to conform to that model.

Another arrival in the Department was Bill Joklik from Canberra, Australia. Bill suggested I look at the work of Laver[6] who used sodium dodecyl sulfate (SDS) to dissociate influenza virion proteins for separation by cellulose acetate strip electrophoresis. I tried dissociating adenovirions with SDS and separating them on the high-pH, discontinuous buffer system[7] using 3–5% polyacrylamide gels containing SDS. Figure 1a shows my first experiment with SDS on a 3% polyacrylamide gel stained with Amido Black. More sensitive detection of protein bands resulted from staining with Coomassie Brilliant Blue[8], a dye originally used in Australia for dying wool that became widespread for gel electrophoresis. But in spite of the interesting adenovirus results, there was a nagging reproducibility problem that I later understood, but which caused me to explore other systems.

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